Nuclear transport: **Randy couples**

Ian G. Macara

The recently solved structures of the Ran GTPase with a Ran-binding domain and with karyopherin-β2 have revealed unusually tight embraces that provide important insights into the mechanism of nuclear transport and the many ways in which common protein folds are adapted to perform very different functions.

Address: Department of Pharmacology, Center for Cell Signaling, University of Virginia, 7196 Hospital West, Box 577, Health Sciences Center, Charlottesville, Virginia 22908, USA.
E-mail: imacara@virginia.edu

Current Biology 1999, 9:R436–R439
http://biomednet.com/elecref/09609822009R0436

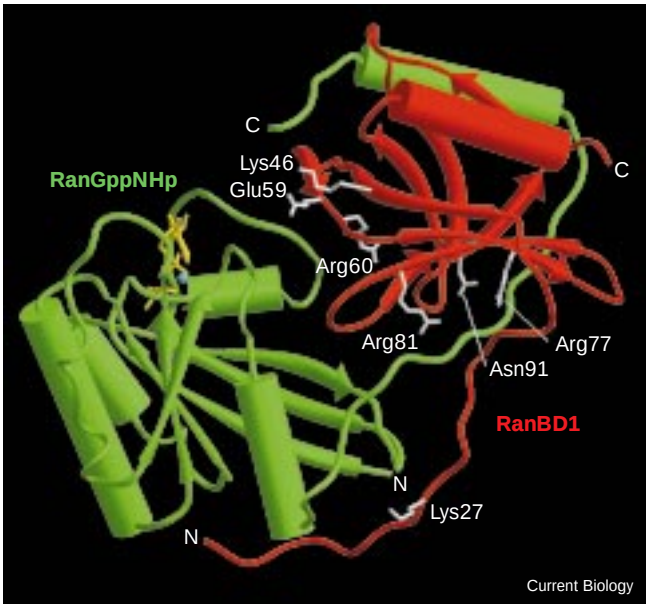
In a reprise of recent events in the White House, the Ran GTP-binding protein has been caught in unusually intimate interactions with two of its many known working partners [1,2]. For a small GTPase, Ran is a very big player, for it drives most forms of macromolecular traffic between the cytosolic and nuclear compartments [3–5]. The defining feature of eukaryotic cells is the compartmentalization of DNA replication and transcription in the nucleus. This separation of functions from translation and other cytosolic activities is achieved by the nuclear envelope, and transport between the nuclear and cytosolic compartments occurs via pores that tunnel through the double shell of the envelope. Cargo is shipped by specific receptors that move through the nuclear pores, probably by facilitated diffusion. The receptors (called importins and exportins or, alternatively, karyopherins) are designed so that, for import, an encounter with RanGTP triggers unloading of the cargo from the importin. Loading of cargo destined for export to the cytosol, on the other hand, is assisted by RanGTP. Cargo is marked for import or export by short signal sequences. The classical nuclear localization signal (NLS) consists of several basic residues often preceded by a proline residue. This NLS is recognized by an adaptor protein called importin-α (or karyopherin-α) which in turn binds (though its 'IBB' domain) to importin-β to form the import complex.

The regulatory factors for Ran — the exchange factor RCC1 and the GTP hydrolysis activator RanGAP — are organized such that RanGTP is produced only in the nucleus and is rapidly destroyed in the cytosol. This arrangement ensures that import cargo can load onto its receptor in the cytosol and will unload only in the nucleus. The empty receptor, bound to RanGTP, must recycle back through the pore to the cytosol, where GTP hydrolysis releases the receptor for another import cycle. In contrast, an export cargo–receptor–RanGTP ternary complex will form only inside the nucleus and, after passing through the pores to the cytosol, is unloaded by hydrolysis of the RanGTP. In this case, the empty receptor presumably cycles back through the pores and into the nucleus on its own [5].

The cytosolic hydrolysis of RanGTP is, therefore, required for both import and export cycles. Yet complexes of RanGTP with importin and exportin receptors are resistant to RanGAP activity [6]. How, then, can receptor unloading or recycling occur? The solution to this problem lies with two proteins, RanBP1 and RanBP2, that function as co-activators of RanGAP — RanBP1 is present in the cytosol and RanBP2 on the outside face of the pores. RanBP1 and RanBP2 contain highly conserved Ran-binding domains that can form ternary complexes with RanGTP and importins/exportins. These ternary complexes are susceptible to RanGAP — and RanBP1 may also release importin–RanGTP complexes from binding sites on the pores, in addition to permitting RanGAP function [7,8].

RanBP1 is a small protein that contains little more than the Ran-binding domain and a nuclear export signal, near its carboxyl terminus, to ensure that it remains cytosolic [9]. RanBP2, on the other hand, is a giant component of

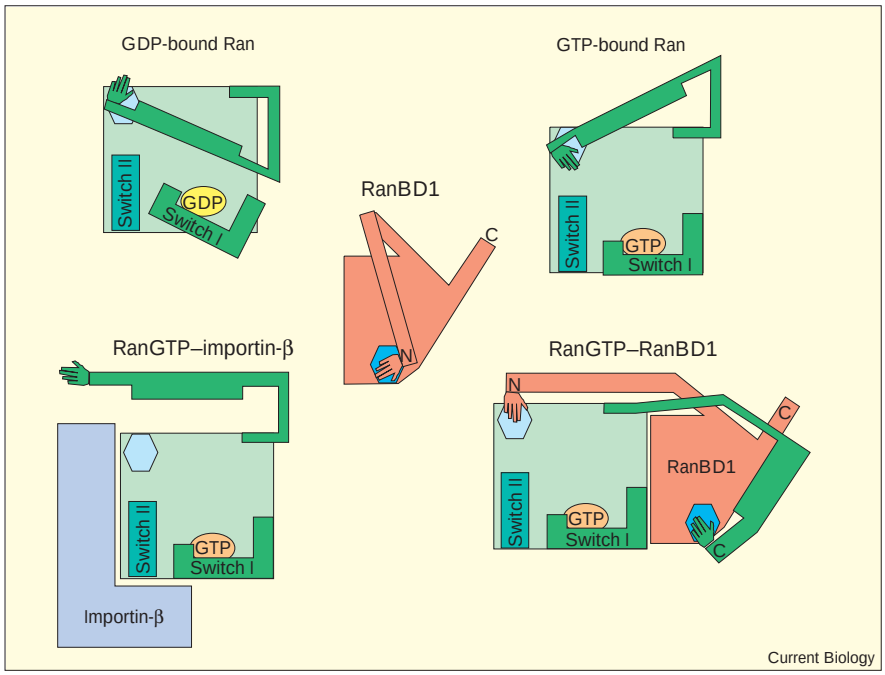
Figure 1



Structure of the RanGppNHp–RanBD1 complex, highlighting several key residues in RanBD1 that are conserved in RanBP3 and that interact with Ran through salt bridges or hydrogen bonds. The guanine nucleotide is shown in yellow and the Mg^{2+} ion in blue.

Figure 2

Cartoon of conformational changes in various Ran complexes. The free RanGTP and RanBD1 structures have not been solved and are speculative. The light blue hexagons represent patches of basic residues, and the hands represent acidic motifs. The carboxy-terminal acidic hand of Ran in the GTP-bound form is proposed to remain associated with the basic patch with which it interacts in the GDP-bound form (residues 139–142), but the carboxy-terminal α helix swings out to permit rotation of the Switch I region upon binding GTP. Association with importin-β displaces the carboxyl terminus of Ran from the basic patch on the Ran surface. In free RanBD1, it is proposed that the acidic amino-terminal region has rotated so as to interact with the same basic area with which the acidic –DEDDDL motif of Ran interacts in the RanGTP–RanBD1 complex (residues Lys46 and Arg130).



fibrils that extend into the cytoplasm from the nuclear pores [10,11]. In addition to several other domains, RanBP2 contains four independent Ran-binding domains and is associated with a modified form of RanGAP. As a result, it probably acts at the front line to release outgoing importin–RanGTP complexes, while RanBP1 and soluble RanGAP function as a backup system.

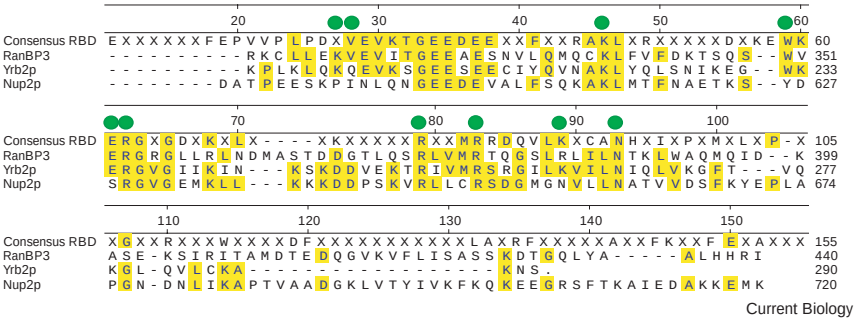
The Ran-binding domains bind with nanomolar affinity to RanGTP, inhibit nucleotide exchange on Ran and co-activate RanGAP. They can also form ternary complexes with the importins/karyopherins plus RanGTP or RanGDP. To further understand this rich biochemistry we need structures, and these have been provided in abundance over the past few months. First came the crystal structure of a Ran-binding domain complexed with Ran in its GTP-bound state, closely followed by structures of importin-β with the IBB domain of importin-α and of karyopherin-β2 (also called transportin) with Ran [1,2,12]. Vetter *et al.* [1] co-crystallized the first Ran-binding domain of RanBP2 (RanBD1) with Ran bound to a non-hydrolyzable GTP analog, GppNHp. Comparison of this complex and of the karyopherin-β2–RanGppNHp complex with the structure of RanGDP also allowed analysis of a novel switch mechanism employed by the GTPase [13]. In addition, the relationship of the Ran-binding domain fold to a host of other domains means that we can make predictions about their structures.

The most striking feature of the complex of RanBD1 with RanGppNHp is the intimate embrace between the two proteins: the amino terminus of RanBD1 hugs Ran, and the carboxyl terminus of Ran is stretched out around RanBD1 so as to almost encircle it (Figure 1). This embrace may be coordinated by acidic 'hands' on the carboxyl and amino termini of Ran and RanBD1, respectively. In the isolated proteins, the hands probably interact with basic patches on their own surfaces. But on forming the complex, the acidic hand at the carboxyl terminus of Ran wraps around to the basic patches on RanBD1, and the acidic amino terminus of RanBD1 reaches over to touch the basic patch on Ran (Figure 2). Thus, the unattached proteins keep their hands in their own pockets, but in the embrace they extend arms to clasp each other.

In addition to hand-holding, the bodies of RanBD1 and Ran are in very close contact. The majority of contacts between the two proteins involve residues in the central region of the Ran-binding domain, particularly the invariant WKER and MRR…CANH motifs (in the single-letter amino acid code; Figure 3). The WKER motif interacts with the effector loop (Switch I) of Ran, near the amino terminus, whereas the MRR…CANH motif interacts mainly with residues in the carboxy-terminal half of the Ran protein.

Comparing the structures of Ran in the GDP-bound and GppNHp-bound conformations, the most dramatic changes occur in the Switch I effector loop and in the carboxyl terminus. Switch I is believed to undergo a conformational change in all small GTPases, thereby permitting interaction

Figure 3



Alignment of the consensus Ran-binding domain (RBD, derived from sequences in RanBP1 and RanBP2 of several species; numbered as in the crystal structure shown in Figure 1) with human RanBP3, a structural homolog from budding yeast called Yrb2p, and the yeast nucleoporin Nup2p. Conserved residues are shown in yellow; green spots identify conserved residues that in RanBD1 interact via salt bridges or hydrogen bonds with RanGppNHp [1].

with downstream target proteins only when in the GTP-bound state. The carboxyl terminus of Ran is unique amongst the known small GTPases in its possession of a long arm terminating in a highly acidic hand (–DEDDDL). In RanGDP, as mentioned above, this hand is most probably attached to the surface through a basic patch (residues 139–142), and this may stabilize the GDP-bound state [12]. The interaction of Ran with GTP forces a change in the conformation of the Switch I loop which displaces the carboxy-terminal arm — it then swings through almost 180° to make contact with RanBD1 (Figure 1).

The structure of RanGppNHp in the karyopherin-β2 complex is quite similar to that in the RanBD1 complex, with the carboxy-terminal arm swung out away from the body of the Ran protein. In the karyopherin-β2 complex, however, the arm is floating free [2]. This conformation had been predicted from the observation that an antibody directed against the –DEDDDL motif recognizes Ran bound to importin-β, but does not recognize free RanGTP [14]. However, another antibody, which recognizes a carboxy-terminal epitope just upstream of the –DEDDDL motif, can recognize free RanGTP but not RanGDP, suggesting that although the carboxy-terminal arm may rotate outwards when Ran changes nucleotide-bound state, the acidic hand remains attached to its basic patch until Ran associates with an importin-β (Figure 2) [15]. Deletion of the –DEDDDL motif increases the rate of importin-β binding to RanGTP, perhaps because it eliminates the need for this conformational change. Ran-binding domains can form ternary complexes with RanGTP and importin-β and can function as co-activators of RanGAP. Do the new structures provide any insights into these processes? First, as expected, the regions of contact of the RanBD1 and of the karyopherin-β2 do not overlap; however, they are adjacent. Karyopherin-β2 is a remarkable structure, comprising a string of so-called HEAT motifs. These motifs consist of an α-helical hairpin that resembles the armadillo repeat found in importin-α and β-catenin. There are 18 HEAT motifs in karyopherin-β2, which form a twisted structure bent in the middle like an elbow. The twist produces a concavity at the amino terminus that binds Ran. In the importin-β structure reported by Cingolani *et al.* [12], a carboxy-terminal concavity binds the IBB domain, snaking around to almost completely enclose it. Whereas RanBD1 binds to one end of the Switch I region of Ran, karyopherin-β2 interacts with the other end (residues 44–47 of Ran) and with Switch II (Figure 2). Karyopherin-β2 also presses close to the central region of Ran, smothering a large area of the surface that in RanGDP is masked by the carboxy-terminal arm. Lying between the two halves of the karyopherin-β2 and the importin-β structures is an acidic loop that in karyopherin-β2 contacts Ran and in importin-β contacts the IBB domain. The loop sequences are different in the two proteins, but it is tempting to speculate that this overlap might be a part of the trigger that releases cargo in the nucleus, where the importin binds RanGTP. The structure of importin-β bound to RanGppNHp will test this idea (A. Wittinghofer, personal communication).

As discussed above, RanGTP bound to importin-β is resistant to RanGAP, which most probably interacts with the Switch I and II regions of Ran. It remains unclear whether RanGAP can interact with RanGTP in a quaternary complex with a Ran-binding domain protein and importin-β, or whether the importin-β must first be released. However, competition between the Ran-binding domain and importin-β for interaction with Ran residues Asn154 and Glu158 could weaken the grip of RanGTP on importin-β, and the binding of importin-α to the carboxy-terminal domain of importin-β would be expected to further loosen its hold on the Ran protein. Once free of the clutches of importin-β, the Ran-binding domain may increase the affinity of RanGTP for RanGAP by holding aside the acidic carboxy-terminal motif, accelerating GTP hydrolysis and terminating the transport cycle.

In addition to RanBP1 and RanBP2, there are two other known proteins that possess ‘partial’ Ran-binding domains: RanBP3 (Yrb2p in budding yeast) and Nup2p (Figure 3). But these proteins are not on intimate terms with Ran — RanBP3 binds RanGTP with only micromolar

affinity (about 10^3-fold lower than the affinity of RanBD1 for RanGTP), and the affinity of Nup2p for RanGTP is unlikely to be higher [16]. The region of closest similarity lies in the amino-terminal half of the Ran-binding domain, but critical sidechains that interact with the effector loop of Ran — such as the lysine (K) in the EWKER motif — are not present (Figure 3). Moreover, the amino terminus of the RanBP3 domain lacks an acidic character, suggesting that its arm may not contact the basic patch on Ran. The automatic threading procedure of the SWISSMODEL server aligned only the first 54 amino acids of the RanBP3 domain with RanBD1; the structure in this region is probably similar to RanBD1. Interestingly, this region corresponds exactly to the side of the molecule that faces Ran. Ten out of 15 hydrogen bond or salt-bridge interactions could be preserved if RanBD1 were replaced by RanBP3 (Figure 3); however, specific interactions to the effector loop of Ran would be reduced. The last two residues of the EWKER motif (which are similar in RanBP3) make contacts only through their peptide backbone atoms. One could speculate that RanBP3 might not be able to discriminate between the effector loops of RanGTP and RanGDP (the carboxy-terminal linker could still hinder the binding, but under certain circumstances it might be displaced).

One surprise is that the Ran-binding domain possesses the same up-and-down β-barrel topology as the pleckstrin homology (PH) and phosphotyrosine binding domains. These sequences possess no detectable similarities to one another. PH domains can bind to phosphoinositide lipids, through a region that corresponds to the area on the Ran-binding domain that interacts with the Ran effector loop (Switch I). The β-barrel fold appears to be very common. It is present in PDZ domains, many of which interact with carboxy-terminal protein motifs, and which are present in at least 50 different proteins [17]. Callebaut *et al.* [18] recently noticed a significant similarity between the first WASP homology (WH1) domain of the WASP family of cytoskeletal proteins and the sequences of the Ran-binding domains. Like PH domains, the WH1 domain of N-WASP is thought to mediate binding of the phosphoinositide PIP_2. Callebaut *et al.* predicted that the conserved sequence stretches within these domains would form β strands, rather than α helices, and it seems very likely that all of the WH1 domains fold into up-and-down β barrels. Indeed, such a structure has just been confirmed for the WH1 domain from the protein Mena [19]. The conserved sequences match closely to β strands of the Ran-binding domain structure, with the exception of the KERG region in the β-strand 2 of RanBD1, which is not conserved amongst the WH1 domain sequences. Clearly the β barrel is a versatile fold for small domains that are designed to interact with both second messenger molecules and proteins.

The exposure of Ran in flagrante delicto with two of its partners has revealed tantalizing insights into the hidden world of nuclear transport, but has also whet the appetite for more. How, for example, do exportins simultaneously bind Ran and cargo? How do transport receptors interact with the nuclear pores, and what conformational change triggers their release? What is the structural basis for cargo selection? Stay tuned.

Acknowledgements

We would like to thank Ingrid Vetter and Fred Wittinghofer for performing the RanBP3 threading, for helpful suggestions and for generously providing Figure 1.

References

1. Vetter IR, Nowak C, Nishimoto T, Kuhlmann J, Wittinghofer A: Structure of a Ran-binding domain complexed with Ran bound to a GTP analogue: implications for nuclear transport. *Nature* 1999, 398:39-46.
2. Chook YM, Blobel G: Structure of the nuclear transport complex karyopherin-β2–Ran·GppNHp. *Nature* 1999, 399:230-237.
3. Corbett AH, Silver PA: Nucleocytoplasmic transport of macromolecules. *Microbiol Mol Biol Rev* 1997, 61:193-211.
4. Mattaj IW, Englmeier L: Nucleocytoplasmic transport: the soluble phase. *Annu Rev Biochem* 1998, 67:265-306.
5. Gorlich D: Transport into and out of the cell nucleus. *EMBO J* 1998, 17:2721-2727.
6. Bischoff FR, Gorlich D: RanBP1 is crucial for the release of RanGTP from importin-β related nuclear transport factors. *FEBS Lett* 1997, 419:249-254.
7. Kehlenbach RH, Dickmanns A, Kehlenbach A, Guan T, Gerace L: A role for RanBP1 in the release of CRM1 from the nuclear pore complex in a terminal step of nuclear export. *J Cell Biol* 1999, 145:645-657.
8. Deane R, Schafer W, Zimmermann HP, Mueller L, Gorlich D, Prehn S, Ponstingl H, Bischoff FR: Ran-binding protein 5 (RanBP5) is related to the nuclear transport factor importin-beta but interacts differently with RanBP1. *Mol Cell Biol* 1997, 17:5087-5096.
9. Richards SA, Lounsbury KM, Carey KL, Macara IG: A nuclear export signal is essential for the cytosolic localization of the Ran binding protein, RanBP1. *J Cell Biol* 1996, 134:1157-1168.
10. Yokoyama N, Hayashi N, Seki T, Pante N, Ohba T, Nishii K, Kuma K, Hayashida T, Miyata T, Aebi U, *et al.*: A giant nucleopore protein that binds Ran/TC4. *Nature* 1995, 376:184-188.
11. Wu J, Matunis MJ, Kraemer D, Blobel G, Coutavas E: Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain, and a leucine-rich region. *J Biol Chem* 1995, 270:14209-14213.
12. Cingolani G, Petosa C, Weis K, Muller CW: Structure of importin-β bound to the IBB domain of importin-α. *Nature* 1999, 399:221-229.
13. Scheffzek K, Klebe C, Fritz-Wolf K, Kabsch W, Wittinghofer A: Crystal structure of the nuclear Ras-related protein Ran in its GDP-bound form. *Nature* 1995, 374:378-381.
14. Hieda M, Tachibana T, Yokoya F, Kose S, Imamoto N, Yoneda Y: A monoclonal antibody to the COOH-terminal acidic portion of Ran inhibits both the recycling of Ran and nuclear protein import in living cells. *J Cell Biol* 1999, 144:645-655.
15. Richards SA, Lounsbury KM, Macara IG: The C terminus of the nuclear RAN/TC4 GTPase stabilizes the GDP-bound state and mediates interactions with RCC1, RAN-GAP, and HTF9A/RANBP1. *J Biol Chem* 1995, 270:14405-14411.
16. Mueller L, Cordes VC, Bischoff FR, Ponstingl H: Human Ranbp3, a group of nuclear RanGTP binding proteins. *FEBS Lett* 1998, 427:330-336.
17. Fanning AS, Anderson JM: PDZ domains: fundamental building blocks in the organization of protein complexes at the plasma membrane. *J Clin Invest* 1999, 103:767-772.
18. Callebaut I, Cossart P, Dehoux P: EVH1/WH1 domains of VASP and WASP proteins belong to a large family including Ran-binding domains of the RanBP1 family. *FEBS Lett* 1998, 441:181-185.
19. Prehoda KE, Lee DJ, Lim WA: Structure of the Enabled/VASP homology I domain peptide complex: a key component in the spatial control of actin assembly. *Cell* 1999, 97:471-480.